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PRELIMINARY INVESTIGATION OF THE STRUCTURE OF THE CARBOHYDRATE COMPONENT OF *Vicia graminea* LECTIN, A PLANT GLYCOPROTEIN*

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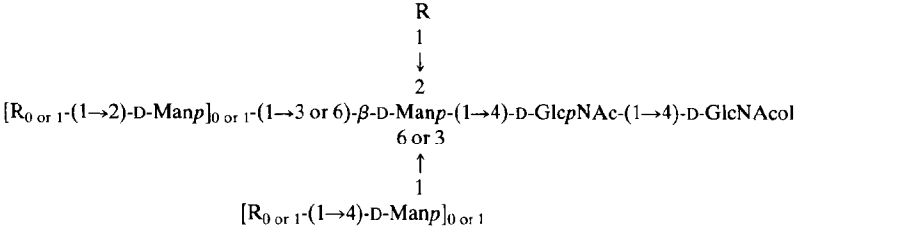
ABSTRACT

Purified *Vicia graminea* lectin, isolated from seeds, was found to contain D-mannose, 2-acetamido-2-deoxy-D-glucose, L-fucose, D-galactose, and D-xylose in the molar ratios ~3.9:1.5:1.2:1.1:1.0. The oligosaccharides, obtained after hydrazinolysis of Vg-lectin, were *N*-reacetylated, reduced with sodium borohydride, and fractionated into two peaks. The first peak contained D-galactose, D-mannose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-glucitol in the molar ratios 6:3:2:0.7. After h.p.l.c. fractionation into five oligosaccharides, the second peak contained D-mannose, D-xylose, L-fucose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-glucitol. Methylation analysis suggested the following general structure for these oligosaccharides:



R = α-D-Man*p* or D-Xyl*p*

INTRODUCTION

Lectins constitute a special group of proteins capable of binding specific carbohydrate structures[1]. The best characterized lectins, in terms of chemical struc-

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ture and carbohydrate-binding properties, are derived from plants. Except for the lectins of *Canavalia ensiformis*[2] (concanavalin A), *Triticum vulgare*[3] (wheat germ agglutinin), *Pisum sativum*[4] (pea), and *Arachis hypogea*[5] (peanut), plant hemagglutinins contain small proportions of carbohydrates[6]. Very little is known of the glycan components of lectins, and only a few of them have been well characterized. The carbohydrate chains mainly contained D-mannose, 2-acetamido-2-deoxy-D-glucose, and smaller proportions of L-fucose[7,8]. Bromelain glycopeptides[9] and some lectins (*e.g.*, tora bean[10] and erythrina[11]) also contain D-xylose.

Vicia graminea, which contains a lectin specific for the N blood-group, has been extensively used for study of red-cell membrane glycoproteins carrying MN blood-group specificity. It was previously reported[12] that *Vicia graminea* lectin (Vg-lectin) purified from seeds was a glycoprotein containing per subunit protein (M_r 25 000) ~5 residues of D-mannose, 2 of 2-acetamido-2-deoxy-D-glucose, and small proportions of D-galactose, D-xylose, and L-fucose, but the structure of its oligosaccharide component was unknown. We report herein a preliminary investigation of the structure, by hydrazinolysis, preparative h.p.l.c., methanolysis, and methylation, of the carbohydrate moiety of Vg-lectin.

EXPERIMENTAL

Analysis. — Carbohydrate analysis of the oligosaccharides and glycoprotein was carried out by g.l.c. after methanolysis and per(trifluoroacetylation)[13].

Hydrazinolysis. — Vg-lectin (8.7 mg), purified from seeds as previously described[10], was dissolved in anhydrous hydrazine[14,15] (0.5 mL), and the solution kept for 24 h at 105°. After evaporation under a stream of nitrogen, the residue was dissolved in saturated sodium hydrogencarbonate solution (1 mL), and acetic anhydride was added in five successive steps (20 μL, every 10 min). After a reaction lasting 2 h, sodium ions were removed by adding Dowex 50-X8 (H^+; 20–50 mesh) cation-exchange resin, and the solution was evaporated *in vacuo*. Oligosaccharides were reduced with sodium borohydride, and, after desalting with Dowex 50-X8 (H^+), boric acid was codistilled with methanol. The reduced oligosaccharides were purified on a Bio-Gel P-2 column (1 × 50 cm). The column was eluted with 2% acetic acid, and fractions were collected (1 mL) at a flow rate of 9 mL/h and analyzed by u.v. absorbance at 206 nm.

Chromatographic procedures. — Oligosaccharides were analyzed by t.l.c. on Silica gel Si 60 (Merck) in 2:1:1 (v/v) 1-butanol–acetic acid–water, with three successive runs. Spots were stained with the orcinol reagent (0.2% orcinol in 20% sulfuric acid). The migration values were compared to those of seven standard oligosaccharides of known structures[16] ($Man_3GlcNAc$–$Man_9GlcNAc$). Preparative liquid chromatography was performed with a Spectra Physics instrument equipped with an Amino AS-5A column (4 × 250 mm, 5 μm, Brownlee Labs Inc., Santa Clara, CA). Chromatographic separation was carried out with a linear gradient from 7:3 to 13:7 of acetonitrile–water for 30 min, followed by isocratic elution for

30 min; the eluted compounds were detected by u.v. absorbance at 200 nm.

Methylation analysis. — Methylation was performed according to Finne *et al.*[17] by an appropriate micromethod. The carbohydrate material (5–20 μg) was dissolved in dimethyl sulfoxide (100 μL), and sulfinyl carbanion (100 μL) added under nitrogen atmosphere. After sonication for 1 h, the solution was frozen and methyl iodide was slowly added (200 μL). The mixture was stirred for 1 h, and the methylated compound extracted three times with chloroform (200 μL each). After being washed with water (5 × 1 mL), the organic phase was evaporated *in vacuo*, and excess dimethyl sulfoxide eliminated by lyophilization. The methylated oligosaccharides were methanolyzed with 1.5M methanolic hydrogen chloride for 17 h at 80°, and the resulting partially methylated monosaccharides were acetylated at 25° with 1:1 (v/v) pyridine–acetic anhydride (50 μL). The partially acetylated and methylated derivatives were identified by g.l.c.–m.s. according to Fournet *et al.*[18], by use of a capillary column (0.35 mm × 60 m) coated with DE-52, and a programmed temperature-rise from 110 to 230° at 4°/min. The methylated derivatives were analyzed and identified by electron-impact (e.i.m.s.), and chemical-ionization mass spectrometry (c.i.m.s.) with a Riber-Mag 10 × 10 instrument (Reuil-Malmaison, France), using ammonia as reactant gas, or by multiple-ion detection.

RESULTS AND DISCUSSION

The Vg-lectin was extracted from seeds cultivated under artificial conditions. After purification, only 10 mg of lectin per kilogram of seeds was obtained, which may explain the absence of earlier structural investigation. The extracted Vg-lectin contained 7% of sugars comprising xylose, fucose, galactose, mannose, and *N*-acetylglucosamine in the molar ratios of 1.0:1.2:1.0:3.9:1.5, together with traces of glucose.

Fractionation of oligosaccharides. — The Vg-lectin (8.7 mg) was hy-

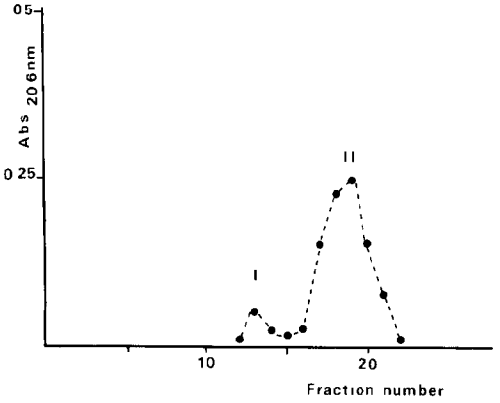


Fig. 1. Gel filtration, on a Bio-Gel P-2 column (1 × 50 cm), of the reduced oligosaccharides released by hydrazinolysis of Vg-lectin (8.7 mg).

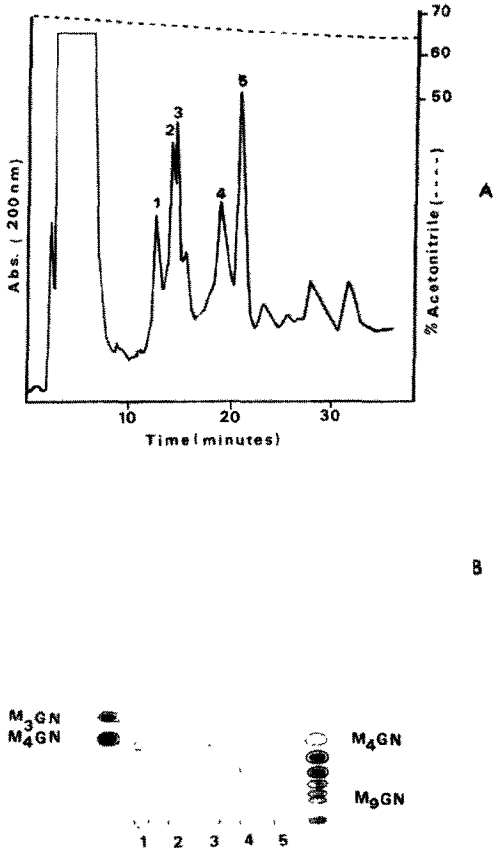


Fig. 2. H.p.l.c. (A) and t.l.c. (B) of Oligosaccharides 1 to 5 (Table I) of Vg-lectin released by hydrazinolysis. Standards: $Man_3GlcNAc$ to $Man_9GlcNAc$[14].

drazinolyzed, and the oligosaccharides released were *N*-reacetylated, reduced with $NaBH_4$, and fractionated on a Bio-Gel P-2 column that gave two peaks (Fig. 1).

Peak I was shown by t.l.c. to contain one compound having a migration rate lower than that of $Man_9GlcNAc$. The yield of 15 μg was determined by methanolysis, which showed that this peak was composed of galactose, mannose, 2-acetamido-2-deoxyglucitol, and *N*-acetylglucosamine in the ratios 6.0:3:0.7:2 (calculated on the basis of 3 mannose residues).

Peak II, which was shown by t.l.c. to be heterogeneous, was fractionated by h.p.l.c. into five oligosaccharides (Fig. 2). They displayed, in t.l.c., migration rates corresponding to those of penta- to octa-saccharides (composition and yield are given in Table I). The yields of Oligosaccharides 1–5 were calculated to be 12, 22, 25, 17, and 30 μg, respectively. All five oligosaccharides contained mannose (3 or 4 residues), *N*-acetylglucosamine (1 residue), 2-acetamido-2-deoxyglucitol (1 residue), and xylose (1 residue), and Oligosaccharides 4 and 5 also included fucose.

TABLE I

CARBOHYDRATE COMPOSITION AND YIELDS OF THE Vg-LECTIN OLIGOSACCHARIDES RELEASED BY HYDRAZINOLYSIS AND FRACTIONATED BY H P.L.C

Oligosaccharides	*Yield (μg)*	*Molar ratios*[a]				
		Fuc	*Xyl*	*Man*	*GlcNAc*	*GlcNAcol*
1	12		1	4.1	1.2	0.75
2	22		1	2.86	1	0.59
3	25		1	4	1	0.59
4	17	0.79	1	3.94	1	0.62
5	30	0.9	1	4.26	1.09	0.67

[a]Determined in the presence of *myo*-inositol as the standard. Ratios based upon 1 mol of Xyl/mol of oligosaccharide.

TABLE II

G L.C DATA FOR PARTIALLY METHYLATED AND ACETYLATED METHYL GLYCOSIDES AND ALDITOLS DERIVED FROM METHYLATED OLIGOSACCHARIDES

Methyl glycosides	*Retention time*[a] *on a SE-52 column*		*Molar ratios for oligosaccharides*				
			1	*2*	*3*	*4*	*5*
	Oligo-saccharide 4[b]	*Authentic compound*[c]					
2,3,4-Tri-*O*-methylxyloside (**a**)	0.54	0.54	+[d]	+[d]	+[d]	+[d]	+[d]
2,3,4-Tri-*O*-methylfucoside	0.64	0.64	0	0	0	0	0
2,3,4,6-Tetra-*O*-methyl-mannoside (**b**)	1 (α) 1.07(β)	1 (α) 1.07(β)	1	1	1	1	1
2-*O*-Acetyl-3,4,6-tri-*O*-methylmannoside (**c**)	1.27	1.27	0.18	0.20	0.32	0.15	0.29
3-*O*-Acetyl-2,4,6-tri-*O*-methylmannoside (**d**)	1.50	1.50	0.30	0.33	0.45	0.31	0.41
6-*O*-Acetyl-2,3,4-tri-*O*-methylmannoside (**e**)	1.55	1.55	0.26	0.39	0.37	0.25	0.35
4-*O*-Acetyl-2,3,6-tri-*O*-methylmannoside (**f**)	1.57	1.57	0.16	0.24	0.45	0.13	0.38
2,6-Di-*O*-acetyl-3,4-di-*O*-methylmannoside (**g**)	1.82	1.82	0.33	0.29	0.44	0.25	0.37
2,3-Di-*O*-acetyl-4,6-di-*O*-methylmannoside (**h**)	1.85	1.85	0.51	0.36	0.60	0.37	0.50
3,6-Di-*O*-acetyl-2,4-di-*O*-methylmannoside (**i**)	2.05	2.05	0.32	0.25	0.38	0.31	0.34
2,3,6-Tri-*O*-acetyl-4-*O*-methylmannoside (**j**)	2.38	2.38	0.17	0.12	0.18	0.19	0.17
4-*O*-Acetyl-2-deoxy-1,3,5,6-tetra-*O*-methyl-2-methylacetamidoglucitol (**k**)	2.56	2.56	0.35	0.27	0.23	0.29	0.25
4-*O*-Acetyl-2-deoxy-3,6-di-*O*-methyl-2-methylacetamidoglucoside (**l**)	2.71(α) 2.94(β)	2.70(α) 2.94(β)	0.40	0.39	0.27	0.33	0.30

[a]Relative to methyl 2,3,4,6-tetra-*O*-methylmannoside. [b]Identical values were obtained for the four other oligosaccharides. [c]See ref. 16. [d]Not calculated (products volatilized in the Ross injector).

The loss of carbonydrate, in relation to the starting material, may have been due to the amounts used by preliminary t.l.c. or h.p.l.c., as well as by the presence of minor oligosaccharides not detected during preparative h.p.l.c.

Methylation analysis. — The gas chromatograms resulting from the methylation procedure were not reliable because only 5–20 μg of material was available. Consequently, the partially methylated and acetylated sugars and hexitols were detected by chemical-ionization (NH_3), mass spectrometry using the multiple-ion detection technique. The ions (M + 1), (M + 18), (M − 31), and [M − (31 + 32)] or [M − (31 + 60)], corresponding to mono-, di-, tri-, and tetra-*O*-methyl derivatives of the Vg-lectin oligosaccharides were monitored. The relative amounts of each derivative were estimated by comparing the "fragmentograms" obtained with those of authentic samples, except for xylose or fucose derivatives, which were volatilized in a Ross injector (Table II). The "fragmentogram" obtained for Oligosaccharide 2 is given in Fig. 3 as an example. Each derivative was identified according to its retention time and, when possible, by e.i.m.s. (Fig. 4). Methyl 2,3,4-tri-*O*-methylfucoside, 4-*O*-acetyl-2-deoxy-3,6-di-*O*-methyl-2-methylacetamidoglucoside, and 2,3,6-tri-*O*-acetyl-4-*O*-methylmannoside were only identified by c.i.m.s. (Fig. 5).

In spite of the apparent homogeneity of the spots observed in t.l.c., subsequent methylation analysis showed that the five oligosaccharides were extremely heterogeneous. At the same time, methylation also gave practically identical results for all five, since it indicated that they had similar structures deriving one from the other by the addition of mannose, xylose, or fucose residues. In any case, they had one main characteristic in common, a mannosyl residue substituted at O-2, -3, and -6 giving methyl 4-*O*-methylmannoside. On the basis of a protein-linked di-*N*-acetylchitobiosyl core[19], the common structure **1** deduced from the methyl deriva-

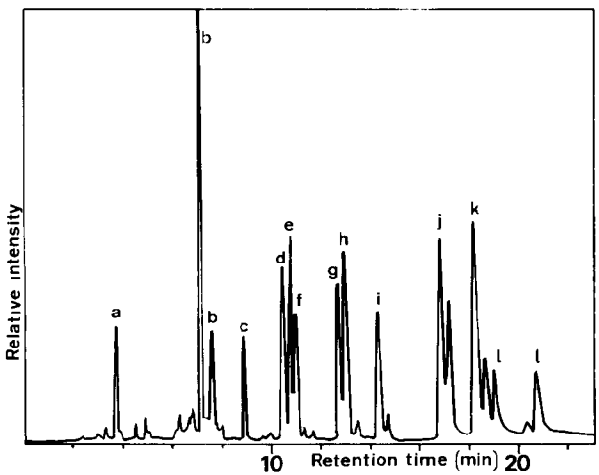


Fig. 3. "Fragmentogram" obtained by methylation of Oligosaccharide 2. The formulas of the methylated derivatives **a–l** are given in Table II.

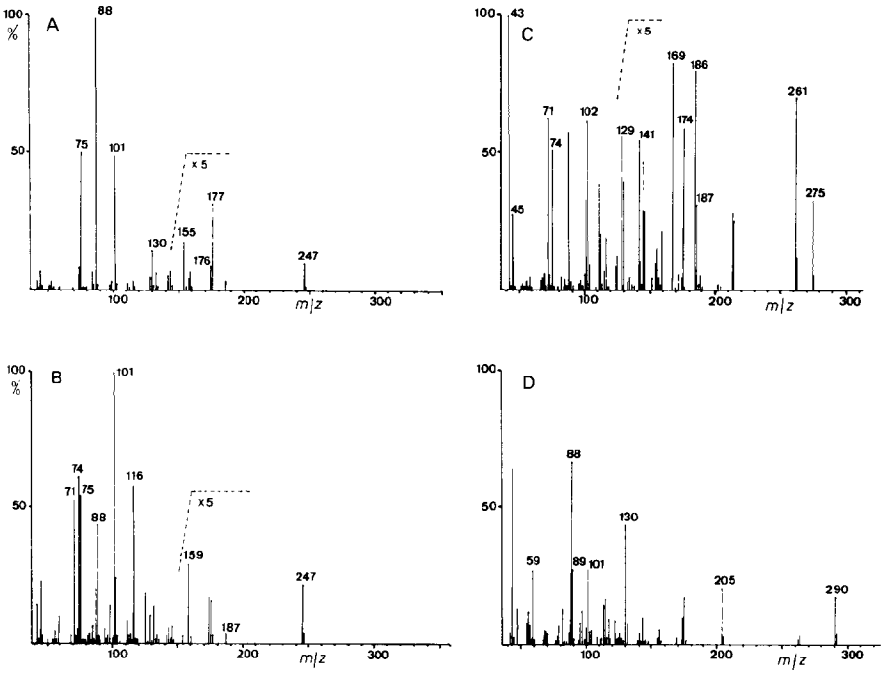


Fig. 4. Mass spectra (e.i.) of methyl glucosides and alditols derived from methylated oligosaccharides: (A) 6-*O*-acetyl-2,3,4-tri-*O*-methylmannoside; (B) 3-*O*-acetyl-2,4,6-tri-*O*-methylmannoside; (C) 2,3-di-*O*-acetyl-4,6-di-*O*-methylmannoside; and (D) 4-*O*-acetyl-2-deoxy-1,3,5,6-tetra-*O*-methyl-2-methylacetamidoglucitol.

tives reported in Table II may be proposed for the Vg-lectin oligosaccharides. This structure is based on the presence of the trisubstituted mannosyl residue, and on methyl tri- and di-*O*-methylmannosides corresponding to the presence or absence of the substituents R, [R-(1→2)-Man], or [R-(1→4)-Man], R being an α-D-mannopyranosyl or D-xylopyranosyl group. The absence of this residue was indicated by methyl 2,4-di-*O*-methylmannoside, but we were unable to determine the position of the xylosyl group. Nevertheless, comparison of **1** with **2** and **3** (bromelain oligosaccharides[9]), and **4** (glycopeptide isolated from the Pronase digest of tora bean[10]) enables us to propose the four probable structures **5–8** for the oligosaccharides corresponding to **1**. Owing to the difficulty of preparing a large quantity of Vg-lectin, these four structures should be considered as preliminary results.

In conclusion, we suggest that oligosaccharide **1** is a mixture of several hexasaccharides representing all the aforementioned combinations. The structures of Oligosaccharides 2–5 (Table I) are probably built on the same model with additional mannosyl and fucosyl residues. These carbohydrate moieties of Vg-lectin exhibited much greater heterogeneity than was observed for other plant lectins[7,10,20]. The interesting features of Vg-lectin glycans are the presence of xylose and fucose, and a 2,3,6-tri-*O*-substituted mannosyl residue. Although these glycan

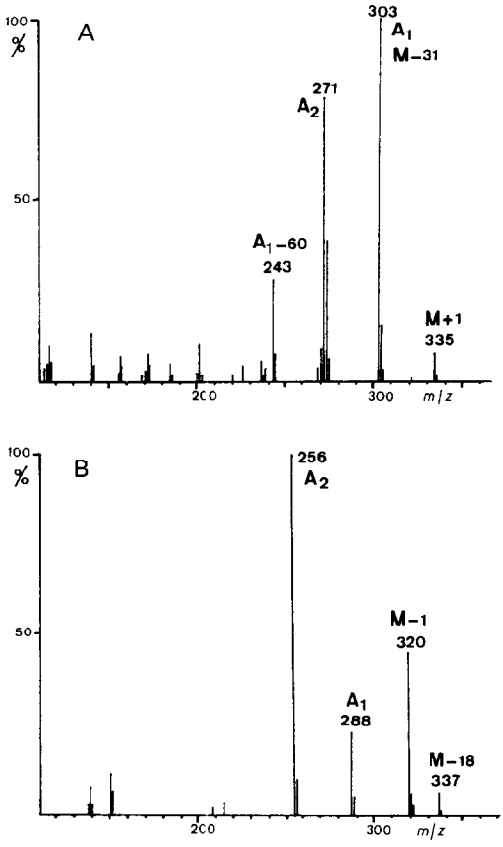
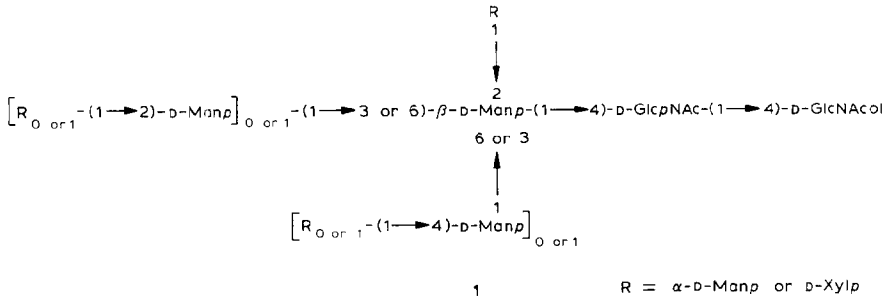
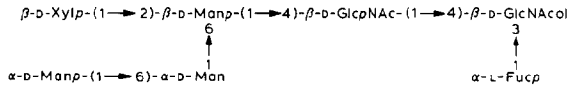


Fig. 5. Mass spectra (c.i., NH_3) of methyl 2,3,6-tri-*O*-acetyl-4-*O*-methylmannoside (A) and 4-*O*-acetyl-2-deoxy-3,6-di-*O*-methyl-2-methylacetamidoglucoside (B).

structures are only preliminary, they may be considered similar to those proposed for other plant glycoproteins, such as bromelain[9] (**2** and **3**) and tora bean lectin[10] (**4**).

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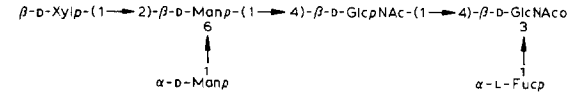
3
1
α-L-Fucp

2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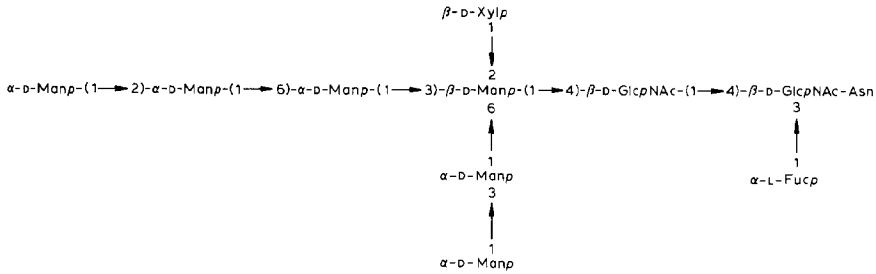
1
α-D-Manp
3
1
α-L-Fucp

3


β-D-Xylp
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2
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6
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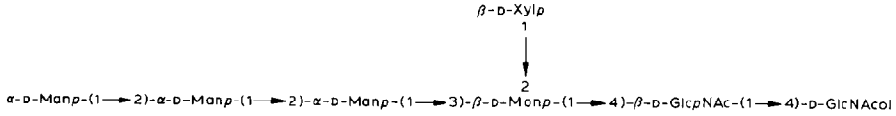
α-D-Manp
3
1
α-D-Manp
3
1
α-L-Fucp

4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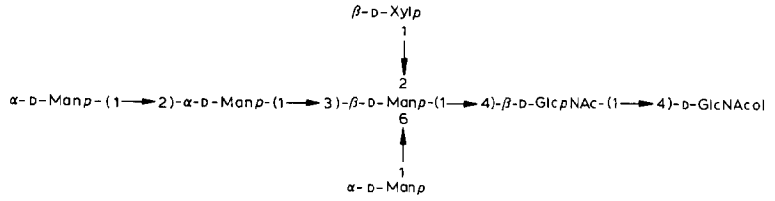
5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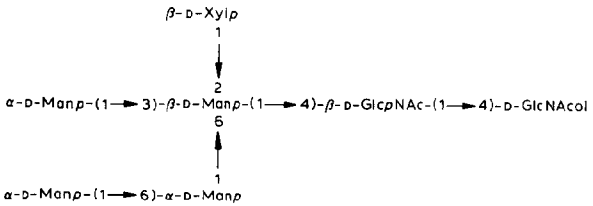
6
1
α-D-Manp

6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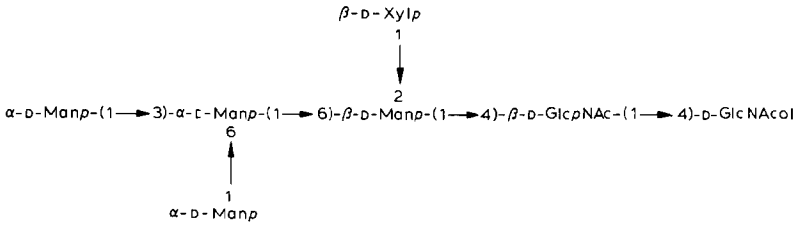
6
1
α-D-Manp-(1→6)-α-D-Manp

7



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